



## Some Indicators of Blood of Rats Poisoned with Pesticide Karate

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### **Abstract**

An artificially reconstructed karate pesticide leads to cytopsy of blood corpuscles and a shift of the Leiko formula to the right. In this case, the erythrocyte indices, the color index of erythrocytes and the average hemoglobin content, as well as the erythrocyte sedimentation rate, significantly increase. A decrease in the number of corpuscles with increased erythrocyte indices (CP and DES), accompanied by granulocytopenia, agranulocyte and accelerated erythrocyte sedimentation rate are additional evidence of B12-deficiency and folate-deficiency anemia. The research of the parameters of peripheral blood poisoned with karate of rats at introduction of its per os RAF is shown that all studied indicators of approximately identical size with indicators of blood of intact rats.

**Keywords:** *Pesticides; Antioxidants; Vegetable Antioxidant Factor; Albumine, Globulins; Leukocytes; Lymphocytes; Monocytes; Peroxide Oxidation of Lipids; Neutrophils; Vegetable Antioxidant Drug; So-So Molecular Peptides; Erythrocytes; Hemoglobin; Thrombocytes; Eosinophils; Cholinesterase*

### **Introduction**

Using it during the long period in agriculture and the industry and in life of different types of the pesticides capable to collect in the soil, foodstuffs, atmospheric air and water created a global problem of environmental control.

Coming to an organism in various ways, pesticides collect in tissues of the person and animals, having on them toxic effect [13, 15, 34]. In cases of disturbances protective functions, there is a need of restoration of the antioxidant status of an organism the preventive or therapeutic purposes. One of the possible ways to solve this problem may be the search for effective antioxidants among natural resources, in particular plants.

Antioxidants – the substances capable in small doses to slow down freely radical oxidation. They are widely applied to prevention of diseases. The researches concerning toxic effect of pyrethroid of karate are conducted with this position at “Botanic” department of Tashkent State Pedagogical University named Nizami within several years. Questions of a resolving of consequences of poisoning by the use received in laboratory of department, the vegetable antioxidant factor (VAF) [1, 2, 3] are studied. However, influence of pesticides on such important system of an organism as peripheral blood, according to the world literature is studied not enough [10,27]. In this regard, a research objective was studying of effect of synthetic peritroid pesticide of karate and also with a combination of a vegetable antioxidant factor on all-clinical indicators of blood.

## **Discussion**

Karate – synthetic peritroid, consisting from chlorinator organic compound, which is widely applied, in agriculture. As objects of a research served white rats males of the line Vistar, weighing about 100 grams.

The pesticide was administered orally as an aqueous suspension through a tube at a dose of 1/10 LD<sub>50</sub> once. After seeding with karate, one group of animals, 30 minutes later, was injected with an alcoholic 5% extract of the plant antioxidant factor in an amount of 1 ml for 4 days. After karate inoculation and RAF administration, all rats were kept on a normal diet. On the 1, 5, 10, 20, 30, 40, 50th days, control and experimental rats were slaughtered for experiments.

In the experiments, we used the blood of rats obtained from the tail according to a well-known method [20]. Determination of blood hemoglobin was carried out by the pogramiglobincyanide method [9]. The determination of the number of erythrocytes in the blood was carried out by the test tube method according to Nikolayev, followed by counting in the Goryaev chamber [16].

The leukocyte formula is calculated in stained blood smears, as well as unified microscopic examination of smears [12]. To determine the color index, the color index of erythrocytes was calculated. The index reflects the relative content of hemoglobin in erythrocytes [14]. The average content of hemoglobin in erythrocytes was defined by division of concentration of hemoglobin in 1 mcl blood on number of erythrocytes in the same volume [16]. Definition of the blood sedimentation rate (BSR) was measured by Panchenkov's method [16].

Blood as highly functional and a morph a dynamic system quickly reacts to various influences of external environment, including to harmful factors [11, 35]. Hb is a blood pigment which role consists in fixing and transfer of oxygen from lungs to fabrics and delivery of carbonic acid from fabrics in lungs. The results of our studies of determining the hemoglobin content in the blood of rats under the action of karate and after the introduction of RAF are shown in Figure 1.

Determination of hemoglobin in the blood of intact rats showed that normally its content averages  $110 \pm 1.8$  g/l.

From the provided data of figure 1, it is visible that in all terms after poisoning of karate of a hemoglobin content norms are lower. On early terms of poisoning (the first 10 days) of karate of rats, there is statistically doubtful decrease in amount of hemoglobin of blood.

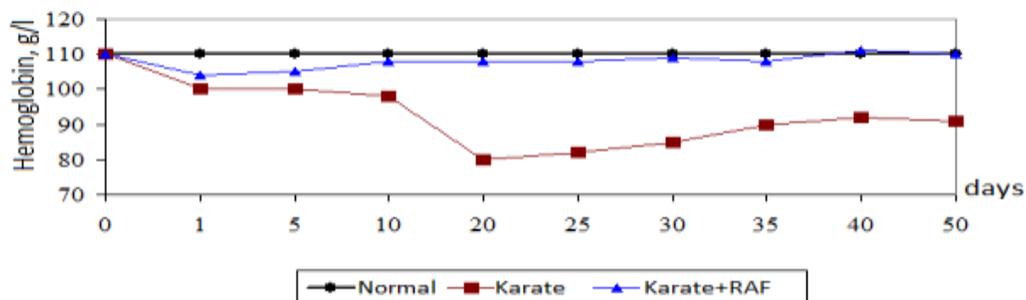
On the 20th, 25th and 30th days of poisoning, the greatest decrease in the amount of hemoglobin is observed, that is, by 27.2; 25.5 and 22.8%, respectively. On the 40th and 50th day of the experiment, the amount is respectively 92 and 91 g/l, which is 16.4 and 17.3% lower than the norm.

As can be seen from the described fact, on the first day of karate pesticide poisoning, the rat blood hemoglobin is at the normal level. The decrease in the amount of hemoglobin, starting from the 20th day, is apparently explained by the fact that the toxic effect of the drug first affects the organ level, and then on the peripheral blood. This is evidenced by the amount of hemoglobin, which is not restored to normal values until the end of the experiment.

When feeding of the rats poisoned with karate RAF, other picture is observed. In this series of experiments within 50 days the hemoglobin content is in norm limits, and there is even slightly more norm in the last decades of a research. Such picture observed in the presence of RAF suggests an idea that intoxication leading to inhibition of erythrocytes of education is suppressed with antioxidant, and at the expense of it, the hemoglobin content in blood is restored.

Our data on the amount of hemoglobin during rat karate intoxication coincide with the data of Shulyak et al. [33], who studied the effect of pesticides - decis, dursban, foxim, copper oxychloride, polycarbacin. They found the suppression of erythropoiesis, which led to a decrease in the amount of hemoglobin over time. Under the influence of these pesticides, the hemoglobin content is not restored even after 30 days.

According to laboratory studies by Lukyanchuk and Luyk [7, 21], the change in blood in persons exposed to dinitrophenol is characterized by a decrease in the content of hemoglobin. These researchers suggest that the decrease in hemoglobin is associated with a decrease in red blood cell synthesis.



**Fig. 1. Changes in the hemoglobin content in the blood of rats under action of karate and karate + RAF. The quantity is taken as the norm hemoglobin of intact rats, equal to  $110 \pm 1.8$  g/l.**

Thus, as can be seen from the above data, the action of the karate pesticide changes the hemoglobin content in the blood after ten days in all the periods studied by us. By changing the content of gamoglobin, synthetic pyrethroid karate affects the functional role, that is, the supply of oxygen to the

cells of tissues and organs changes. Our research results indicate that RAF exhibits a protective effect when administered after karate poisoning.

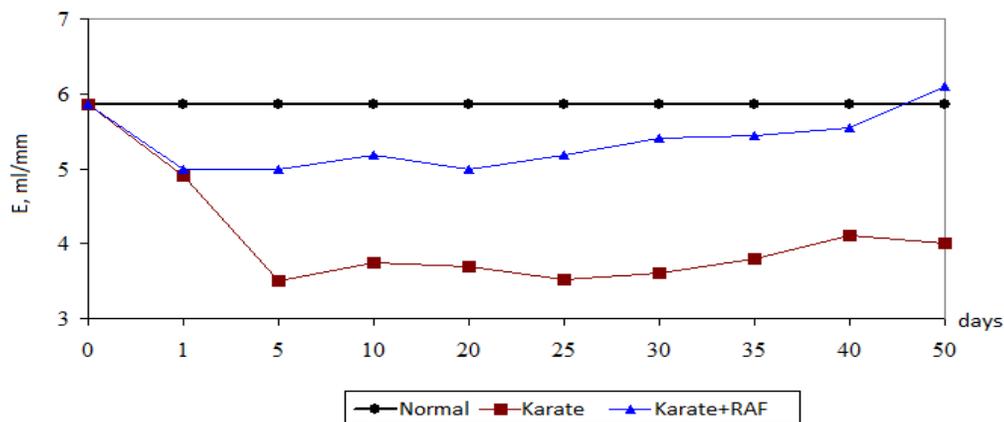
In our next experiments, it was studied uniform elements of blood of the rats poisoned with karate. Erythrocytes - the most numerous uniform elements of blood which main maintenance makes hemoglobin. [14].

Results of quantity determination of erythrocytes of blood of the rats poisoned with karate showed that these uniform elements undergo certain changes (rice 2.).

Apparently, from figure 2, in a day erythrocytes begin to decrease considerably and with increase in term of poisoning, the difference between indicators of experimental and intact rats increases. Continuous decrease in quantity of erythrocytes reaches the maximum difference on 10 – 25 days of an experiment. So for 5, 10, 20 and 25<sup>th</sup> days of poisoning of karate of rats decrease in quantity of erythrocytes on 40,2, 46, 46,8 and 40%, respectively is defined. Further, 30-, 35-, 40 and 50-days, and makes 38,5, 35, 30 and 31,7%, respectively, a little reduce the difference between control and experiences.

Considering the data on the number of erythrocytes and hemoglobin, it can be assumed that the karate pesticide in the body of poisoned rats disrupts metabolic processes, as a result of which anemia develops. On the other hand, a decrease in the number of erythrocytes and hemoglobin may be according to our assumption, a consequence of a violation of erythropoiesis at the stage of reticulocyto formation. Apparently, these changes lead to the development of anemia.

With the introduction of RAF into the body of rats poisoned with the karate pesticide, a significant change in the number of erythrocytes is observed within 25 days after poisoning. Under the influence of RAF, the number of erythrocytes, reduced to 40-46% in rats poisoned by karate, decreases relative to the norm to 11.6-14.6%. In the rest of the experiment, that is, on days 30, 35, 40 and 50, the number of erythrocytes is almost at the level of the norm and is  $109\pm 3.0$ ;  $108\pm 3.4$ ;  $111\pm 2.8$  and  $110\pm 3.0$  g/l (Fig. 2).



**Fig 2. Change in the number of erythrocytes in rats under the action of karate and karate + RAF. Taken as the norm erythrocyte count of intact rats equal to  $5.85\pm 0.29$**

In other terms of an experiment, quantity of erythrocytes are almost up to the standard 46%, in the rats poisoned with karate, the quantity of erythrocytes is reduced concerning norm to 11.6 - 14.6%. In

other terms of an experiment, that is 30-, 35-, 40 and 50-days the quantity of erythrocytes is almost up to the standard and is  $109\pm 3,0$ ;  $108\pm 3,4$ ;  $111\pm 2,8$  and  $110\pm 3,0$  of g/l (fig. 2).

Analyzing the results of which determining the number of erythrocytes and hemoglobin, it can be concluded that RAF, used by a single injection for 5 days, sufficiently normalizes the processes of erythrocyte formation, disturbed by the pyrethroid karate.

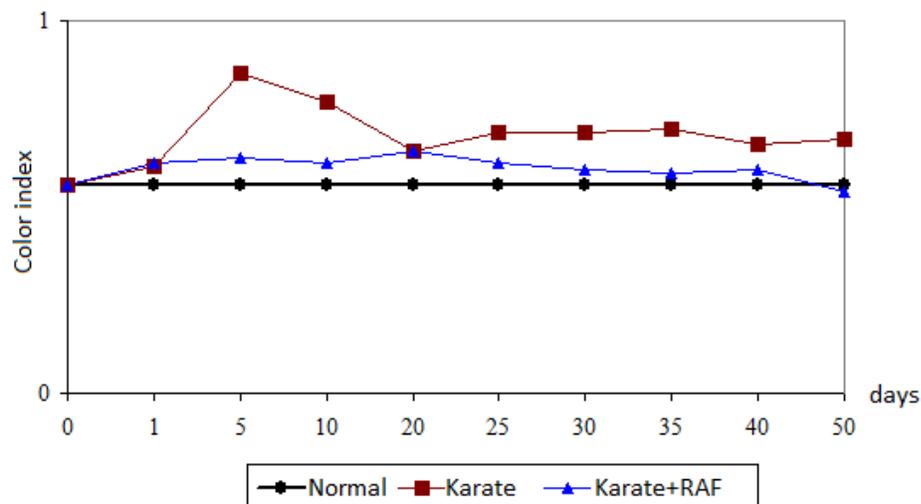
To check our assumption of possible development of anemia under the influence of karate, calculated a color indicator of erythrocytes and average content of hemoglobin in erythrocytes. In size of a color indicator, it is accepted to divide anemias into hypochromia, normochromy and hyperchromic.

The obtained data of a color indicator of erythrocytes are provided in a type of a curve in figure 3. Apparently, from figure 3, throughout all terms of an experiment the color indicator of erythrocytes is higher than norm. For the 5 and 10 day of poisoning of karate, the greatest increase color an indicator of erythrocytes is observed. In other days, the raised indicator is found too, and the smallest difference between a control and skilled indicator for the 20th day equals 16%.

When determining the average content of hemoglobin in erythrocytes, it was found that in all periods of the experiment under the influence of karate, this index is increased. If we compare the index of the color index of erythrocytes and the average content of hemoglobin, then an almost identical picture is obtained.

As in the case of the color index of erythrocytes and the average hemoglobin content when feeding rats with RAF, the increased index decreases and approaches the norm. This suggests that the RAF has a positive effect on the ongoing changes caused by karate.

According to literary data [20,22,26], it is known that increase in a color indicator of erythrocytes and average content of hemoglobin – a hyperchromia – is characteristic laboratory sign various  $B_{12}$  – deficient and folic deficiency [nutritional, alimentary] anemia.



**Fig. 3. Color index of erythrocytes in the blood of rats under action of karate and karate + RAF. Average is taken as the norm erythrocyte index of intact rats equal to  $0.56\pm 0.2$**

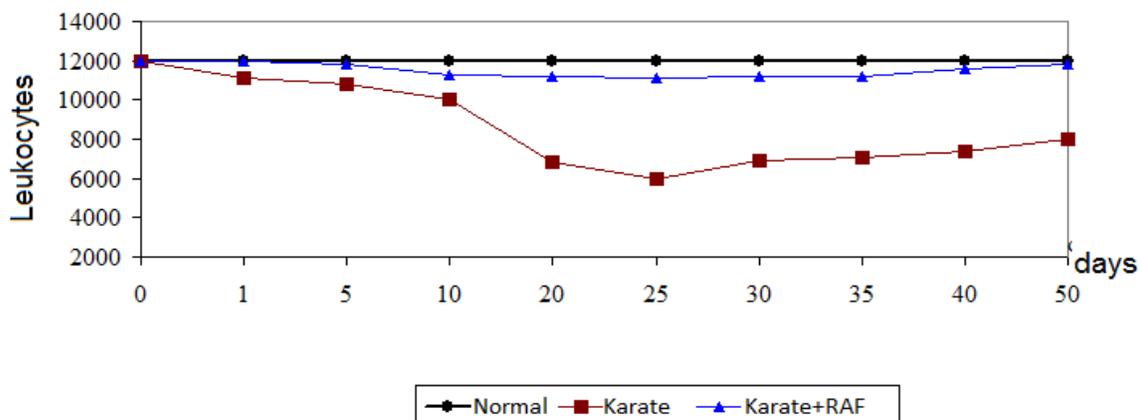
If to consider the above data, then it is appropriate, to provide earlier obtained data on the fact that when opening a peritoneum of rats discoloration of a mucous membrane of guts was observed. They were plentifully covered with black slime that was not observed at intact rats [18,28]. It, probably, led to disturbance of functions of absorbability of intestines. Therefore, for this reason absorption of B<sub>12</sub> vitamin is broken.

It is known that leukocytes (L) are colorless blood cells of various functions in animals and humans. All types of leukocytes (basophils, eosinophilic, neutrophils, lymphocytes and monocytes) have a nucleus and methods for active amoeboid movement. The body absorbs bacteria and dead cells, produces antibodies [11, 14].

Since our goal was to study the peripheral blood, it goes without saying that it is necessary to study the leukocyte content of the formed elements. The results received when determining quantity of leukocytes are given in figure 4. From the scheme, it is visible that at the rats poisoned with karate the quantity leukocytes gradually decrease and then sharp lowering is observed. Usually reduction in the amount leukocytes is called a leukopenia. The leukopenia is aggravated, since 20th day and for 20-, 25-, 30th days decreases on 43.3; 50; 42.5%% from norm, and in further terms of poisoning, that is for the 35-, 40-, 50 days the difference between control and experience in the content of quantity of leukocytes decreases a little and makes 40.8; 38.3 and 33.3%.

It can be seen from the graph in Figure 4 that the curve of the leukocyte content of rats fed with RAF is similar to the curve of leukocytes of intact animals. Significant differences in the number of leukocytes are not determined between these lines. Decrease in quantity of erythrocytes, hemoglobin, increase in a color indicator of erythrocytes, average content of hemoglobin, reticulocytopenia, leukopenia, neutropenia, shift of a blood count to the right, increase a blood sedimentation rate is observed at persons with deficiency in an organism of B<sub>12</sub> vitamin which leads to development of megaloblastic, hyper chromic, macrocytic anemia, thrombocytopenia, a neutropenia and damage of nervous system like funicular myelosis [20,28,29].

Since in our experiments a similar picture is observed, that is, a decrease in erythrocytes, hemoglobin, an increase in the color index of erythrocytes, the average content of hemoglobin, leukopenia, we need to study the definition of such indicators as platelets, leukocyte formula and erythrocyte sedimentation rate.

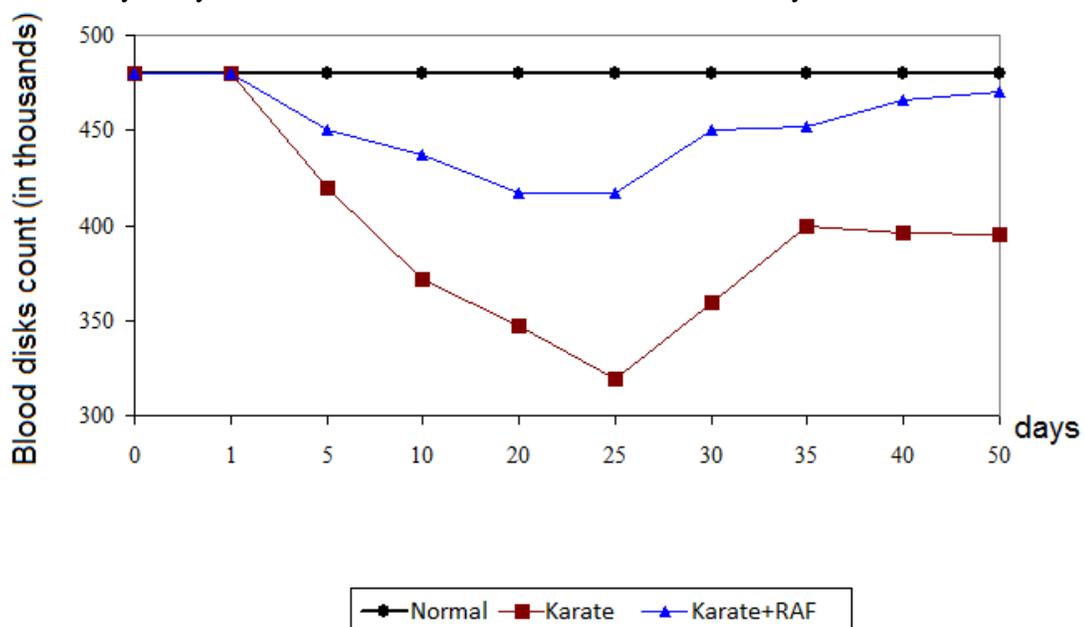


**Fig. 4. Change in the number of leukocytes in the blood of rats under the influence of karate and karate + RAF. The number of leukocytes is taken as the norm intact rats equal to 12000**

Based on the foregoing in the following experiments, we investigated the effects of karate and RAF on platelets. Platelets, otherwise called platelets or Bizzocero's plaques, are not true cell formations, but are detached parts of the protoplasm of bone marrow megacaryocytes and have a size of 1.2-2.0 microns. Plates of plate - possess antigenic properties and their main role is participation in hemostasis [11, 19].

Blood disks were determined by the well-known Fonio method [9]. The results are shown in Figure 6. The overall picture gives an idea of thrombocytopenia. The decrease in the number of blood disks, which began on the 5th day of poisoning throughout the entire experimental period, does not return to the control value. On the 10th, 20th, 25th and 30th days of poisoning, a decrease in the number of blood disks by 22.5 is found; 27.7; 33.5 and 25 %%, respectively. On the 35th, 40th and 50th day, the number of blood disks remains almost unchanged and amounts to  $400\pm 37$ ;  $396\pm 25$  and  $395\pm 27$  thousand per thousand erythrocytes.

On one thousand erythrocytes of blood of intact rats, 480 thousand thrombocytes are returned to normal.



**Fig. 5. The blood disks count (in thousands) in the blood of rats under action of karate and karate + RAF. The quantity is taken as the norm blood disks in intact rats, equal to  $480\pm 17$ .**

Also in figure 5, it is observed that when feeding the RAF indication of thrombocytes are close to norm that says this antioxidant drug adjusts the happening changes at karate poisonings. Revealed, in the analysis provided on figure 5, the picture of thrombocytopenia confirms existence of anemia at the rats poisoned peritroid karate.

Thrombocytopenia associated with the action of toxic substances is manifested by bleeding of varying degrees, and hemorrhagic syndrome is accompanied by anemization of the body [4, 7]. To confirm the occurrence of  $B_{12}$  - deficiency anemia under the toxic effect of karate, the leukocyte blood count was studied in detail, as well as the erythrocyte sedimentation rate, the results of which are shown in the figures in the following experiments.

In the following studies, the effect of karate on the leukocyte formula was studied.

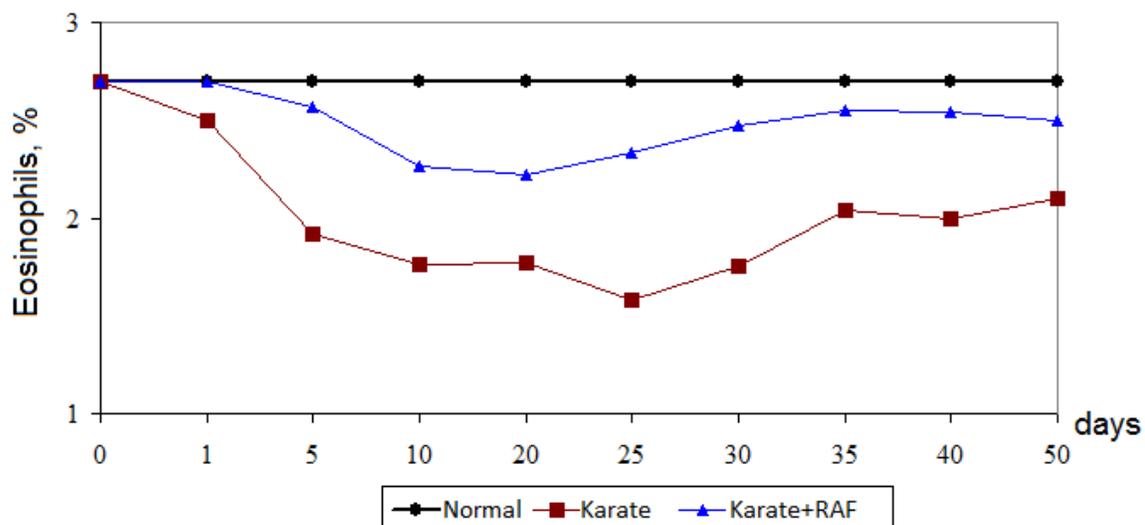
Leukocyte formula is the percentage of various forms of leukocytes in the blood [14]. Since the changes in this formula may be typical for a certain disease, we set ourselves the task of investigating the changes that occur in the leukocyte blood count when rat karate is poisoned. To do this, we determined the percentage of granulocytes: eosinophils, stab neutrophils and segmented neutrophils; agranulocytes: lymphocytes, monocytes.

It is known that [5,17,30,35] toxic substances, in particular, pesticides can act as haemo depressors which interfere with processes of peroxide oxidation of lipids, DNA synthesis, oxidizing phosphorylation that also reduction of survival of cells results in defect of products, first of all - granulocytes.

Counting the number of eosinophils gives the picture shown in Figure 6. The figure shows that in intact rats,  $2.7 \pm 1.3\%$  of eosinophils are determined. When rats are poisoned with pyrethroid karate, a general decrease in the content of eosinophils is observed. Therefore, a 7.5% decrease in the number of eosinophils is determined on the first day of poisoning. On the 5th, 10th, 20th, 25th and 30th days of the study, the content of eosinophils decreases by 29, respectively; 34.8; 34.4; 41.5 and 35.2%. On the 35th, 40th and 50th day, these indicators are close to each other and the difference between the number of eosinophils in the control and experimental blood decreases and is 24.4; 26.9 and 22.2%.

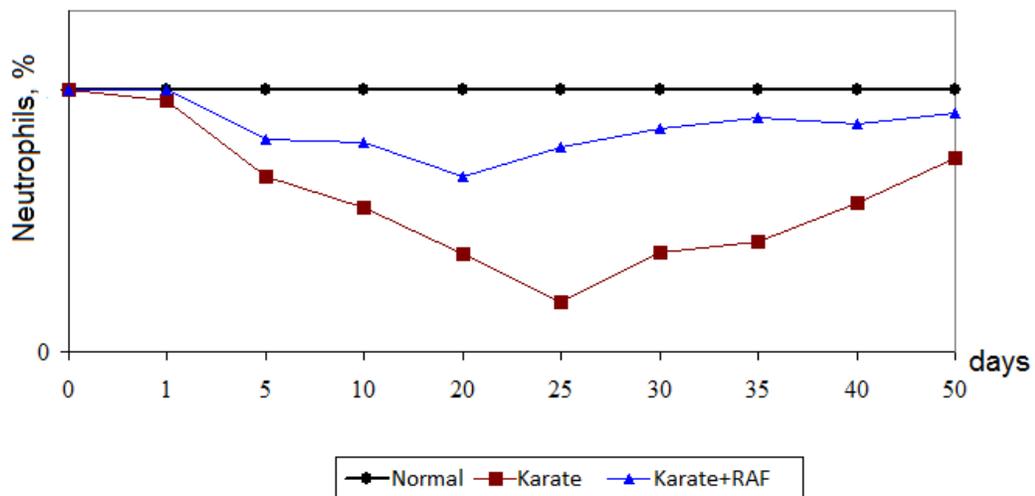
RAF injected into poisoned karate rats helps bring the number of eosinophils closer to normal. On the 20th day, the greatest difference was found between the eosinophils of the intact and karate + RAF variants, equal to 17.8%, versus 41.5% in the poisoned karate rats.

Further determination of the number of stab and segmented neutrophils gives approximately the same picture as eosinophils (see Fig. 6, 7 and 8).

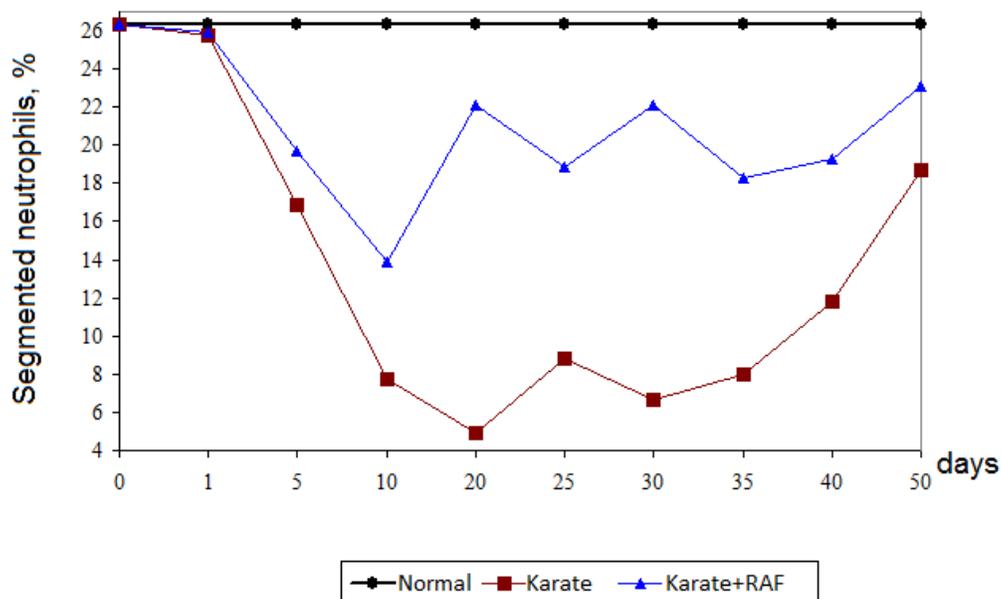


**Fig. 6. The content of eosinophils in the blood of rats under the influence of karate and karate + RAF. The quantity is taken as the norm eosinophils of intact rats, equal to 2.7%.**

Thus, for 20-25 days of poisoning the total number of granulocytes averages 6.68% of quantity of leukocytes whereas in intact and stimulated RAF options the general maintenance of granulocytes equals to 30.0 and 25.2% of the general leukocytes respectively.



**Fig. 7.** Content of stab neutrophils in the blood of rats under the influence of karate and karate + RAF. The norm was taken as the number of stab neutrophils in intact rats, equal to 1%.



**Fig. 8.** The content of segmented neutrophils in the blood of rats under the influence of karate and karate + RAF. The number of segmented neutrophils of intact rats, equal to 26.3%, was taken as the norm.

Depression degree of manifestation eritro-, leucio- and a thrombocytopoiesis depends on intensity and duration of action of an etiological factor, individual sensitivity of an organism to its action, conditions of the internal causes influencing a hemogenesis, in particular at women (the demonstrating or latent deficiency of iron, dysfunction of thyroid, thymic glands, ovaries, etc.).

Thus, causing considerable reorganizations in structure and metabolism of fabrics, cells and subcellular structures, pesticides are nonspecific structural and metabolic poisons.

Considering that studies of the effect of pesticides at the molecular level are not always possible to reproduce, and even more so, to be carried out for preventive purposes, then it is necessary to develop available methods of research and prevention from the consequences of pesticide poisoning.

Still, it was considered [4.26] that changes of blood at influence of pesticides is the integrated indicator reflecting all-toxic action on an organism that is, pesticides have no direct impact on the system of a hemopoiesis. However, in literature of steel data about development of hematologic diseases, such as, polycarce, pernicious, hypo - and aplastic anemias, agrunolocytosis, myeloblastosis [6, 8, 23, 24, 25, 31, 32] meet at the people working in contact with pesticides and for those who accidentally got poisoned with high doses in case of accidents.

Studies of the peripheral blood parameters of rats poisoned by karate when administered per os with RAF show that all the studied parameters are approximately the same as the blood parameters of intact rats.

The above results of our studies give reason to believe that the RAF used as an antioxidant has a positive effect, noticeably (almost the same as the norm) bringing peripheral blood readings closer to the norm.

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